REMARKS

Claims 41-69 are pending and have been examined in the present Office action. Claims 42-50, 54-56, 58-68, and 70-81 were rejected under 35 U.S.C. § 112, first paragraph, for failure to comply with the written description requirement and for an asserted lack of enablement. Claims 43 and 62-65 were also rejected under 35 U.S.C. § 102(b). Claims 44, 45-48, 50, 56, 58, 62-66, 70-73, 75, and 81 were provisionally rejected under the judicially created doctrine of obviousness-type double patenting. Each of these rejections is addressed below.

The Invention

Pre-eclampsia is a syndrome that affects 5 to 10% of pregnancies and results in substantial maternal and fetal morbidity and mortality. sFlt-1 (the soluble form of the Flt-1 receptor) binds VEGF and PlGF. Applicants have discovered that the levels of sFlt-1 are increased and the levels of free PlGF and free VEGF are decreased in subjects having or at risk for developing pre-eclampsia or eclampsia as compared to a normal reference sample or level. Further, Applicants have discovered that the ratio of the levels of sFlt-1, free PlGF, or free VEGF, relative to each other, are altered in subjects having or at risk for developing pre-eclampsia or eclampsia as compared to a normal reference sample or level. From these discoveries follow the claimed methods for diagnosing pre-eclampsia or eclampsia or a propensity to develop pre-eclampsia or eclampsia, including specific subsets of pre-eclampsia and eclampsia such as early

onset pre-eclampsia or eclampsia. The methods include measuring the level of sFlt-1, free PlGF, or free VEGF polypeptides in a sample from a subject that is the first, second, or third trimester of pregnancy wherein an increase in the level of sFlt-1 or a decrease in the level of free PlGF or free VEGF as compared to a normal reference sample or level is diagnostic of pre-eclampsia or eclampsia or a propensity to develop pre-eclampsia or eclampsia, including early onset pre-eclampsia or eclampsia.

Amendments to the Claims

As an initial matter, Applicants thank Examiner Dang and Primary Examiner Bunner for the productive interview conducted on September 6, 2007. Applicants have amended the claims as discussed during the interview. In addition, as requested, we review below matters discussed in the interview.

Claims 1-40, 43, 51-53, 57, and 69 are cancelled. Claims 41, 42, 44-45, 47, 48, 49, 50, 58-62, 64, 68, and 70-72, were amended for clarity or to correct dependencies. Support for these amendments is found throughout the specification and the claims.

Claim 42 has been amended to recite that the subject is less than 16 weeks pregnant. Support for this amendment is found throughout the specification and the claims, for example in previous claim 42, and at page 38, lines 16-17; page 44, lines 13-16; page 45, lines 21-27; page 47, lines 19-26; and page 48, lines 9-11.

Claim 65 has been amended to remove reference to amniotic fluid.

New claims 82-91 have been added. Independent claim 82 recites a method of diagnosing pre-eclampsia or eclampsia that includes measuring the level of free PlGF in a serum sample and sFlt-1 in a bodily fluid sample, where a level of free PlGF less than 400 pg/ml serum and a level of sFlt-1 greater than 2 ng/ml is diagnostic of pre-eclampsia or eclampsia or a propensity to develop the same. Support for this claim is found throughout the specification and the claims, for example, in previous claims 41, 43, and 50, and at page 49, lines 6-7 and page 53, lines 6-9.

New claims 83 and 84 recite specific isoforms of VEGF and PlGF. Support for these claims is found throughout the specification and the claims, for example, at page 4, lines 24-26; page 18, lines 1-3; and page 23, lines 3-8.

New claim 85 recites monocytes as a type of leukocyte. Support for this claim is found in pending claim 81.

New claims 86 and 87 depend from claim 45 and recite the limitation that the subject is less than 16 weeks (claim 86) or 17-20 (claim 87) weeks pregnant. Support for these claims is found throughout the specification and the claims, for example, at page 44, lines 13-16; p. 45, lines 21-27; page 47, lines 19-26; and page 48, lines 9-11.

New claim 88 depends from claims 41, 45, 49, 50, 80, and 82, and recites the limitation that the diagnosis occurs prior to the onset of at least one symptom of pre-eclampsia or eclampsia. Support for this claim is found throughout the

specification, for example, at page 3, lines 5-6; page 21, lines 19-23; page 44, lines 16-24; page 47; lines 19-26; and page 51, lines 27-29.

New claims 89-90 depend from claims 41, 42, 45, 48, 49, and 50, and recite the limitation that the method diagnoses the subject as having pre-eclampsia or eclampsia (claim 90) or a propensity to develop pre-eclampsia or eclampsia (claim 89). Support for these claims is found throughout the pending claims.

New claim 91 depends from claims 45, 50, and 80 and recites the limitation that the subject is 23-32 weeks pregnant and an increase of at least 50% in the sFlt-1 level or a decrease of at least 50% in the free VEGF or free PlGF level relative to a reference diagnoses the subject as having a propensity to develop early onset pre-eclampsia or eclampsia. Support for this claim is found throughout the specification and the claims, for example, at page 45, line 12 to page 46, line 2.

No new matter has been added by these amendments.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 42-50, 54-56, 58-68, and 70-81 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description and enablement requirements. Each of the rejections is addressed below.

Written Description

Claims 42-50, 54-56, 58-68, and 70-81 stand rejected under § 112, first paragraph, on the assertion that the claims contain subject matter that was not

described in the specification in such a way as to reasonably convey to one skilled in the relevant art that Applicants, at the time the application was filed, were in possession of the claimed invention. Applicants respectfully submit that, in view of previous amendments to the claims in combination with the extensive knowledge in the art at the time of filing, as summarized below, this rejection can be withdrawn.

The written description requirement may be met by (MPEP § 2163; emphasis added) "a disclosure of sufficiently detailed, relevant identifying characteristics which provide evidence that applicant was in possession of the claimed invention...." Moreover, compliance with the written description requirement does not require that information well known in the art be recited in the specification of the instant application. This fact is specifically noted in *Capon v. Eshhar* (Nos. 03-1480, -1481. Fed. Circ. 2005), which states (emphasis added):

> Precedent illustrates that the determination of what is needed to support generic claims to biological subject matter depends on a variety of factors, such as *the existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or technology*, the predictability of the aspect at issue, and other considerations appropriate to the subject matter.

Applicants submit that the specification, when combined with the extensive knowledge in the prior art, is sufficient to satisfy the written description requirements with regard to free VEGF and free PlGF polypeptides, or fragments thereof, that are capable of binding to sFlt-1, as presently claimed.

According to Applicants' model, during pre-eclampsia, levels of sFlt-1 are increased and can complex to PlGF and VEGF, thereby reducing the levels of free PlGF and free VEGF in a subject having pre-eclampsia or eclampsia. The increase in sFlt-1 is associated with pre-eclampsia or eclampsia in both a correlative and causative manner because it is the increased levels of sFlt-1 that are acting as a "physiologic sink" to deplete the levels of free PlGF and free VEGF. The currently rejected claims recite diagnostic methods that include the detection of sFlt-1, free PlGF, or free VEGF in a sample from the subject and include the limitation that the free PlGF and free VEGF detected *have the ability to bind to sFlt-1.* It is the ability to bind to sFlt-1 that is the common attribute and characteristic that defines the members of the claimed genus. This characteristic is described throughout the specification, for example, at page 2, lines 18-24; page 3, lines 9-12; page 4, lines 22-25; page 20, lines 13-16; page 27, lines 15-22; page 28, lines 1-6; and page 46, lines 22-26.

Isoforms and fragments of VEGF and PlGF polypeptides that are capable of binding to sFlt-1 were known to those skilled in the art at the time of filing, as were the amino acids required for binding. Applicants recite below nine prior art references (as cited during the presentation made in the interview with Examiner Dang and Primary Examiner Bunner), included in the Information Disclosure Statement provided herewith or in a previously submitted Information Disclosure Statement, demonstrating that VEGF and PlGF isoforms that bind to sFlt-1 were known at the time of filing of the present application as were the binding domains

of both the sFlt-1 receptor and the VEGF and PlGF ligand. These prior art references and their teachings are summarized below and speak to the extent and content of the prior art and the maturity and depth of the field of research surrounding VEGF and PlGF.

Knowledge in the prior art at the time of filing

- sFlt-1 is a soluble form of the Flt-1 receptor which lacks the transmembrane and cytoplasmic domains. (Kendall et al., *Proc. Natl. Acad. Sci.* 90:10705-10709 (1993))

- sFlt-1 can bind to VEGF and PlGF with an affinity comparable to the membrane bound form of the receptor. (Dvorak et al., *J. Clin. Oncol.* 20:4368-4380 (2002) and Ferrara et al., *Nature Medicine* 9:669-676 (2003))

- The ligand-binding domain of Flt-1 includes the second and third immunoglobulin like domains conserved between Flt-1 and sFlt-1. (Davis-Smyth et al., *EMBO J.* 15:4919-4927 (1996) and Wiesmann et al., *Cell* 91:695-704 (1997))

- VEGF-A (including isoforms VEGF121, VEGF165, and VEGF189), VEGF-B, PlGF-1, and PlGF-2 bind to Flt-1 and sFlt-1. (Dvorak et al., *J. Clin. Oncol.* 20:4368-4380 (2002), Park et al., *J. Biol. Chem.* 269:25646-25654 (1994), Olofsson et al., *Proc. Natl. Acad. Sci.* 95:11709-11714 (1998), and Ferrara et al., *Nature Medicine* 9:669-676 (2003))

- Receptor-binding domains of VEGF were identified through alanine

scanning mutagenesis and resolution of the crystal structure of the VEGF/Flt-1 complex. Critical amino acid residues of VEGF required for binding include amino acids 63-67 (Asp63, Glu64, and Glu67). (Keyt et al., *J. Biol. Chem.* 271:5638-5646 (1996))

- Receptor binding domains of PlGF were identified through alanine scanning mutagenesis and modeling using the crystal structure of PlGF and the crystal structure of the VEGF/Flt-1 complex. Critical amino acid residues of PlGF required for binding include Asp72, Glu73, Gln27, Pro98, and Tyr100. (Iyer et al., *J. Biol. Chem.* 276:12153-12161 (2001))

- An alignment of VEGF and PlGF shows conservation of the overall structure of the proteins and of the receptor binding domains. (Iyer et al., *supra*)

In sum, Applicants submit that the binding of VEGF and PlGF polypeptides, including isoforms and fragments thereof, to sFlt-1 was very well characterized at the time of filing, as were the structural features of both the ligand (VEGF and PlGF) and the receptor (sFlt-1) required for binding. In addition, as demonstrated by the ample literature characterizing the interaction in other contexts between a variety of VEGF and PlGF isoforms, fragments, and mutants and the Flt-1 or sFlt-1 receptor prior to the time of filing of the present application, Applicants submit that the methods of readily testing the receptor binding ability of a VEGF or PlGF polypeptide were clearly well-known in the art at the time of filing.

Applicants submit that one of skill in the art would, at the time of filing,

understand that the ability to bind to sFlt-1 is the common attribute and characteristic that defines the members of the claimed genus and would recognize that the requirements for VEGF and PlGF binding to sFlt-1 were well-known in the art. Applicants submit that the written description requirement has been met and respectfully request that the rejection of claims 42-50, 54-56, 58-68, and 70-81 under 35 U.S.C. § 112, first paragraph, with regard to written description, be withdrawn.

Applicants note that the newly added independent claims 82 includes the limitation that the free PlGF is a PlGF polypeptide that has the ability to bind to sFlt-1. Therefore, for the reasons provided above, this claim would also satisfy the written description requirement.

Enablement

The Examiner sets forth four different reasons for the rejection of claims 42-50, 54-56, 58-68, and 70-81 for lack of enablement. Applicants traverse this rejection in the context of the present amendment and submit that the application meets the enablement requirement with regard to the claims as amended herein.

Amniotic fluid

In the first basis for the rejection, the Examiner rejects claims 66 and 81 for an asserted lack of enablement because the specification does not reasonably provide enablement for measuring sFlt-1, VEGF, or PlGF in amniotic fluid.

Applicants note that the recitation of amniotic fluid pertains to claim 65 and not claims 66 and 81. As proposed during the interview, Applicants have removed reference to amniotic fluid in claims 65. This basis for the rejection can now be withdrawn.

Endothelial cells, leukocytes, monocytes, and cells derived from the placenta

In the second basis for the rejection, the Examiner rejects claims 66 and 81, because the specification does not reasonably provide enablement for measuring PlGF, sFlt-1 or VEGF levels in endothelial cells, leukocytes, monocytes, and cells derived from the placenta. In view of the amendments to the claims and the data provided in the previously submitted Declaration of Dr. Karumanchi and the additional data submitted in the attached Declaration of Dr. Karumanchi, Applicants submit that this basis for the rejection can be withdrawn.

The independent claims, as amended herein, can be broadly grouped into two categories: methods of diagnosing pre-eclampsia or eclampsia by determining the *absolute* threshold level of sFlt-1, free PlGF, or free VEGF, or metrics including these polypeptides or methods of diagnosing pre-eclampsia or eclampsia by determining the *relative* level of sFlt-1, free PlGF, or free VEGF, or metrics including these polypeptides as compared to a reference sample or level. Amended claim 66, from which claim 81 depends, depends only from the claims that recite methods that include determining the relative level of sFlt-1, free PlGF, or free VEGF as compared to a reference sample (i.e., claims 45 and 50 of the

second category described above).

As attested to during the interview, the methods for detecting sFlt-1, free VEGF, and free PlGF described in the specification can be readily applied to the detection of these polypeptides in a cell or tissue sample and would not constitute undue experimentation.

Moreover, in the previously submitted Declaration of Dr. Karumanchi, Applicants provide a working example of the detection and comparison of the levels of sFlt-1 in monocytes from a pre-eclamptic patient and a normotensive patient using the methods described in the specification. The data provided with the Declaration demonstrated that sFlt-1 levels were increased in monocytes from a pre-eclamptic subject as compared to a normotensive control subject.

Applicants submit herewith a second Declaration of Dr. Karumanchi providing another working example of the detection and comparison of the levels of sFlt-1 in a claimed cell type. For this example, the relative level of sFlt-1 in a placental trophoblast cell (i.e., a cell derived from the placenta), from a pre-eclamptic patient and a normotensive patient was determined and compared using the methods described in the specification. The resulting data demonstrate that sFlt-1 levels are increased in placental trophoblasts from six pre-eclamptic subjects as compared to six normotensive subjects.

Applicants reiterate that claim 81, which recites the various cell types, only depends from claims 45 and 50 which require a relative alteration in the levels of sFlt-1, free VEGF, or free PlGF for diagnosis of pre-eclampsia or eclampsia or a

propensity to develop pre-eclampsia or eclampsia. The data provided demonstrate the relative increase in sFlt-1 in both monocytes and placental trophoblasts from pre-eclamptic subjects as compared to normal subjects. A skilled artisan would understand that the same methods can be applied any cell type or tissue type in which the polypeptide is expressed. Applicants submit that this basis for the enablement rejection of claims 66 and 81 can be withdrawn.

Correlation between the structure of PlGF and the ability to bind sFlt-1

In the third basis for the rejection, the Examiner rejects claims 42-50, 54-56, 58-68, and 70-81 for an asserted lack of enablement because the specification, while enabling for PlGF with accession number P49763, does not reasonably provide enablement for the measurement of isoforms and fragments of PlGF. In rendering this rejection, the Examiner states that Applicants have not provided a correlation between the structure of PlGF with its ability to bind to sFlt-1. In view of the previous amendment and the arguments presented above in response to the written description rejection, Applicants submit that this basis for the enablement rejection can be withdrawn.

As an initial matter, Applicants note that the Examiner includes a similar rejection for isoforms and fragments of VEGF but the case set forth for this rejection pertains to therapeutic methods which are not claimed in the present application. Nonetheless, Applicants have addressed the rejection as it pertains to VEGF in the arguments presented below.

Claims 42-50, 54-56, 58-68, and 70-81 feature methods of diagnosing pre-eclampsia or eclampsia that include the measurement of sFlt-1, free VEGF or free PlGF in a sample from a subject. Applicants have previously amended the claims to recite the limitation that the free PlGF is a PlGF polypeptide that *has the ability to bind to sFlt-1* and that the free VEGF is a VEGF polypeptide that *has the ability to bind to sFlt-1*. Applicants submit that PlGF and VEGF isoforms or fragments capable of binding to sFlt-1, were known in the art at the time of filing as were the domains of PlGF, VEGF, and sFlt-1 required for the ligand-receptor interaction.

To satisfy the enablement requirement of 35 U.S.C. § 112, first paragraph, the specification must disclose at least one method for making and using the claimed invention that bears a reasonable correlation to the scope of the claims (M.P.E.P. § 2164.01 (b)). Applicants have met this burden.

The present invention is based on Applicants' discovery that levels of sFlt-1, a receptor for VEGF and PlGF, are increased in subjects with pre-eclampsia or eclampsia. When the levels of sFlt-1 are increased, sFlt-1 can complex with increasing amounts of free PlGF and free VEGF, thereby reducing the levels of free PlGF and free VEGF (see, for example, page 3, lines 9-18). The ability of PlGF and VEGF to bind to sFlt-1 is a critical characteristic of PlGF or VEGF family members useful in the claimed methods and this activity is described throughout the specification (see additional citations from the specification provided above).

As described in detail above in response to the written description rejection,

VEGF and PlGF polypeptides, including isoforms and fragments thereof, that are capable of binding to sFlt-1 were known in the art at the time of filing. In addition, the ligand-binding domains of Flt-1 and sFlt-1 and the receptor-binding domains of VEGF and PlGF were well-characterized in the art at the time the application was filed, as were assays for determining binding of VEGF or PlGF polypeptides to sFlt-1.

It is well-established that a patent need not teach, and preferably omits, what is well known in the art. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987); and *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984).

Using the knowledge in the art and the disclosures in the specification, a skilled artisan could readily determine if a VEGF or PlGF polypeptide is capable of binding to sFlt-1.

With regard to the Examiner's point that some of the PlGF isoforms include a heparin-binding domain while some do not and that the absence of the heparin-binding domain causes the PlGF to exist as a diffusible form, Applicants submit that, as described above, both PlGF-1 (lacking heparin-binding domain) and PlGF-2 (having heparin-binding domain) were known in the art to bind to Flt-1 and sFlt-1. Therefore, the presence or absence of a heparin-binding domain does not affect the binding of these PlGF isoforms to sFlt-1.

In view of the previous amendments to the claims and the arguments presented herein, Applicants submit that this basis for the enablement rejection should be withdrawn.

Human pregnant subjects

In the fourth basis for the rejection, the Examiner has rejected claims 41, 45-50, 54-56, 62-68, and 70-81 for an asserted lack of enablement for non-pregnant human subjects. As proposed during the interview, Applicants have amended independent claims 41, 42, 44, 45, 48, 50, and new claim 82 to recite "human pregnant subject." All of the remaining claims depend from each of these claims. This basis for the rejection can be withdrawn.

Rejections under 35 U.S.C. § 102(b)

Claims 43 and 62-65 remain rejected under 35 U.S.C. § 102(b) as being anticipated by Charnock-Jones, WO 98/28006. The Examiner maintains the rejection on the basis that Charnock-Jones teaches the detection of significantly lower concentrations of PlGF in pre-eclamptic women as compared to healthy controls and that the PlGF taught by Charnock-Jones would inherently possess the same functional and structural attributes as the free PlGF recited in claims 43 and 62-65.

Applicants have cancelled claim 43 in the present amendment. Claims 62-65 have been amended to remove dependency from claim 43. In view of this

amendment, the rejection of claims 43 and 62-65 for anticipation by Charnock-Jones under 35 U.S.C. §102 (b) be withdrawn.

Novelty of New Claims

Of the new claims, only claim 82 is independent. This claim recites a method of diagnosing a human pregnant subject as having, or having a propensity to develop, pre-eclampsia or eclampsia that includes measuring the level of free PlGF in a serum sample and the level of sFlt-1 in a bodily fluid sample. The claim recites the limitation that that a level of sFlt-1 greater than 2 ng/ml is diagnostic of pre-eclampsia or eclampsia or a propensity to develop the same. As described previously, Charnock-Jones presents a model that is the opposite of Applicants' model with respect to sFlt-1 levels in pre-eclampsia. Charnock-Jones does not anticipate amended new claim 82 because, according to Charnock-Jones, the levels of sFlt-1 are *decreased* in pre-eclampsia.

All of the remaining new claims depend from claims that are novel over Charnock-Jones and therefore, the dependent claims, by definition, are also novel over Charnock-Jones.

Rejections under the Judicially Created Doctrine of Obviousness-Type Double Patenting

Claims 44, 45-48, 50, 56, 58, 62, 63, 64, 65, 66, 70-73, 75, and 81 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1, 9, 12, 13, 44, 47, 48, and 54-59 of co-pending U.S. Patent Application Serial No. 11/019,559. Applicants will file a terminal disclaimer, if appropriate, upon an indication of otherwise allowable subject matter.

CONCLUSION

Applicants submit that the claims are now in condition for allowance and such action is respectfully requested.

Enclosed are a Petition to extend the period for replying to the Office action for three months, to and including November 1, 2007, and a check in payment of the required extension fee. Also enclosed is a check for $875.00 in payment of the excess claims fees.

Applicants note that the U.S. Patents Documents on the Form PTO 1449 that was submitted with an Information Disclosure Statement filed on March 21, 2006 were not initialed when the initialed Form PTO 1449 was returned to Applicants. Applicants hereby request that it be initialed and returned with the next Office action.

If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: October 24, 2007

Kimya J. Harris

Kristina Bieker-Brady, Ph.D.
Reg. No. 39,109

Kimya F. Harris, Ph.D.
Reg. No. 57,172

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045